

REMARKS

Applicants appreciate the courtesy shown by Examiner Mummert in discussing this case with Applicants' representatives on August 11, 2010. The discussions of the interview are reflected in the following remarks. A copy of the materials (Technical Explanation) discussed during the interview is attached as well. Further to the Response filed on June 17, 2010, claims 8-83 have been canceled without prejudice or disclaimer. Claims 1-7 are pending.

Claim 1 recites a first primer that contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence (hereinafter, the first primer will be referred to as a "turn-back primer" or "TP" as indicated on page 2 of the Technical Explanation). Claim 1 further recites a second primer that contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other (hereinafter, the second primer will be referred to as a "folded primer" or "FP" as indicated on page 2 of the Technical Explanation). The features of the primer set that includes the TP and FP as recited in claim 1 are explained as follows.

During an isothermal amplification reaction using the primer set according to claim 1, the sequence Ac' on the 3' end portion of the TP hybridizes to a sequence A on a target nucleic acid sequence (the sense strand as shown on page 3 of the Technical Explanation), and a complementary strand of the target acid sequence is synthesized. Then, the sequence B' of the TP can hybridize to a sequence Bc on the newly synthesized complementary strand so as to form a loop, as illustrated on pages 3 and 4 of the Technical Explanation and Figure 3a (a)-(b) of the present specification. The FP has a sequence Cc' on the 3' end portion that hybridizes to a sequence C on the 3' end portion of the antisense strand, and during an amplification reaction, a complementary strand of

the antisense strand is synthesized. In this case, since FP has the folded sequence (D-Dc') on the 5' end portion that hybridize to each other, unlike the sequence B of the TP, the folded sequence does not hybridize to the newly synthesized complementary strand of the antisense strand (see, e.g., the last page of the Technical Explanation).

Details of the downstream reaction mechanism of the amplification reaction using the primer set according to claim 1 are illustrated schematically on pages 4-5 of the Technical Explanation and Figures 3a and 3b of the present specification. As shown on page 4 of the Technical Explanation, during the synthesis reaction, an intermediate having a loop on the TP binding end as shown in Figure 3a (g) is formed. The loop includes a sequence A that allows a new TP to hybridize to the loop, thereby allowing a polymerase to begin elongating the sense strand, as shown in Figure 3a (h). However, as shown in Figure 3b (j)-(k), a new TP cannot hybridize to the folded sequence (D-Dc') formed in the intermediates as shown in Figure 3a (j)-(k). Advantageously, the reaction mechanism involved during isothermal amplification using the primer set according to claim 1 allows single nucleotide polymorphisms (SNPs) to be detected by improving significantly the signal-to-noise ratio as compared to when only TPs are used.

In particular, TPs in general are strong engines for amplification due to the loop formation on the elongated strand during amplification. When a TP that is specific to the target nucleic acid sequence and a TP that is specific to the complementary sequence of the target nucleic acid sequence ("TP-TP primer set") are used, a loop that allows a new TP to hybridize to the loop is formed on both ends of the elongated strands during the amplification reaction.

In the case where a one base pair mismatch is present between one of the TPs and the elongated strand, exponential non-specific amplification still can occur because even though one end of the elongated strands form a mismatched loop as shown on page 7 of the Technical Explanation, a non-mismatched loop that allows a non-mismatched TP to bind is still present on the other end of the elongated strands. The non-mismatched loop can serve as an engine for amplification, and thereby allow exponential non-specific amplification to occur.

On the other hand, when a TP-FP primer set in accordance with claim 1 is used, one end of the elongated strands likewise forms a mismatched loop as shown on page 7 of the Technical Explanation, and on the other end of the elongated strands, a folded portion is formed and prevents the TP from binding. Therefore, exponential non-specific amplification seen with a TP-TP primer set does not occur, and accordingly, the background noise (non-specification amplification) can be reduced and the signal-to-noise ratio can be increased significantly.

Moreover, in general, a TP-TP primer set is difficult to design, as many factors must be considered. For example, when designing the TP-TP primer set, four distinct sequences must be considered in view of the particular template sequence that is to be amplified. Another difficulty with the design of the TP-TP primer set is that successful amplification usually depends on both TPs being functional. That is, even if one TP is functional, the success of the amplification is controlled by the non-functional TP. Even further, when using the TP-TP primer set, the length of the template sequence is limited by the fact that there must be no overlap between the hybridizing regions of the stems of the loops of the elongated strands.

In contrast, the TP-FP primer set in accordance with claim 1 includes a folded sequence that can be designed independently from the template sequence. Moreover, only three distinct sequences must be considered in view of the particular template sequence that is to be amplified. Further, successful amplification is dependent upon only one TP being functional. Even further, the length of the template sequence is not limited by the need to avoid overlap between the hybridizing regions of the stems of the loops as in the case where the TP-TP primer set is used.

Rabbani may disclose a TP as recited in claim 1, but does not disclose a primer set that includes the TP and the FP as recited in claim 1.

In particular, the rejection refers to Figure 1, step 3 of Rabbani for the FP. However, Figure 1, step 3 shows the elongated strand that is formed from a TP. As described above, when the TP in accordance with claim 1 is used, the sequence Ac' on the 3' end portion of the TP hybridizes to a sequence A on a target nucleic acid sequence, and a complementary strand of the target acid sequence is synthesized. Then, the

sequence B' of the TP can hybridize to a sequence Bc on the newly synthesized complementary strand, which is on the 3' side of the Ac' sequence, so as to form a loop.

In Figure 1, steps 1-3 of Rabbani, sequence B' and sequence Ac' of the TP as recited in claim 1 correspond to sequences C and B', respectively, of Rabbani's TP primer, sequence A on the target nucleic acid sequence of claim 1 corresponds to sequence B of Rabbani's target nucleic acid sequence, and sequence Bc on the complementary sequence of the target nucleic acid sequence of claim 1 corresponds to sequence C' on the complementary sequence of the target nucleic acid sequence of Rabbani. Thus, it is clear that the strand that forms the loop structure as shown in Figure 1, step 3 of Rabbani is the elongated strand that is synthesized from binding of the TP, and therefore, the elongated strand having the loop formation is not a primer. In no way does Rabbani teach or suggest the FP as recited in claim 1, let alone a primer set that includes TP and FP as recited in claim 1, or the advantages that such a primer set provides. Accordingly, claim 1 and its dependent claims are patentable over Rabbani.

In view of the foregoing, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.



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